REMARKS

Reconsideration and allowance are respectfully requested. Claims 40-58 are pending.

To establish a case of *prima facie* obviousness, all of the claim limitations must be taught or suggested by the prior art. See M.P.E.P. § 2143.03. A claimed invention is unpatentable if the differences between it and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art. *In re Kahn*, 78 USPQ2d 1329, 1334 (Fed. Cir. 2006) citing the legal standard provided in *Graham v. John Deere*, 148 USPQ 459 (1966). The *Graham* analysis needs to be made explicitly. *KSR v. Teleflex*, 82 USPQ2d 1385, 1396 (2007). It requires findings of fact and a rational basis for combining the prior art disclosures to produce the claimed invention. See *id.* ("Often, it will be necessary for a court to look to interrelated teachings of multiple patents . . . and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue"). The use of hindsight reasoning is impermissible. See *id.* at 1397 ("A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning"). Thus, a rejection under Section 103(a) requires "some rationale, articulation, or reasoned basis to explain why the conclusion of [*prima facie*] obviousness is correct." *Kahn*, 78 USPQ2d at 1335; see *KSR*, 82 USPQ2d at 1396. An inquiry should be made as to "whether the improvement is more than the predictable use of prior art elements according to their established functions." *Id.* at 1396. But a claim which is directed to a combination of prior art elements "is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *Id.* at 1396. Finally, a determination of *prima facie* obviousness requires a reasonable expectation of success. See *In re Rinehart*, 189 USPQ 143, 148 (C.C.P.A. 1976).

There exists a wealth of evidence which supports the contention that the instant invention is not obvious, and that a skilled person would not have been motivated to apply the incubation conditions described by Hagiwara to the teachings of Miyahira, nor

to the combined teachings of Surcel and Sørensen. Furthermore, even if their disclosures are combined, the instant invention is not achieved. This evidence comprises:

a) the limited creativity of one of ordinary skill in the immunological arts;
b) that person's understanding of the meaning of particular immunological terms in and around 1996 when Applicants' invention was made;
c) the state-of-the-art in immunology in and around 1996 when Applicants' invention was made, as it related to the mechanism of activation of the T cell receptor by cognate antigens, and a 'prejudice' that supported long incubation times for T cell activation;
d) the clearly defined scope of the instant invention, in that it relates solely to the enumeration *in vitro* of a primary immune response following pre-sensitization of T cells to antigen *in vivo*;
e) the fact that Applicants' invention is underpinned by several concepts, which were previously discussed and are discussed below and in the attached Annex 1, the key ones of which cannot be derived from any of the immunological art cited by the Examiner (the cited art clearly teaches Concept 5, that is, that the ELISPOT assay is a sensitive method for detecting cytokine secretion from antigen-stimulated cells, but this was a concept well-known from many sources at the priority date);
f) the not only inadequate but, in one case, conflicting teachings in the documents cited to support an alleged *prima facie* case of obviousness; and
g) the fact that the invention represents an important immunological advance, and supports a significant commercial success, and has been granted in Europe.

These points are elaborated on below.

The Skilled Artisan

U.S. case law indicates that one of ordinary skill in the art is likely to be aware of all of the pertinent art; ***think along the lines of conventional wisdom in the art***; and above all, be unable to innovate, by which is meant the carrying out of patient syste-

matic research or be in possession of extraordinary insights. See *Standard Oil v. American Cyanamid*, 7734 F.2d 448, 454 (Fed. Cir.1985). *KSR v. Teleflex* characterizes the same person as "a person of ordinary creativity" and "not an automaton," and Applicants submit that such a person in the immunological arts would not have been capable of generating important concepts because they were not derivable from the prior art by that person.

In view of the above, Applicants establish below that their claimed invention would not have been made in and around 1996 by one of ordinary skill in the art with any reasonable expectation of success.

A Skilled Person's Understanding of the Immunological Arts in the Time Period 1993-1996

A. The Phrases "Peptide-Specific T Cell" and "Antigen-Specific T Cell"

By way of introduction, much was known by 1996 about the relevant mechanisms involved in the primary immune response mediated by T cells. For example, an article entitled "T Cell Activation Pathways" (Wingren *et al.*, Crit. Rev. Immunol. 15:235-253, 1995) describes the first signal to the T cell as being the occupancy of the T cell receptor by the antigen/MHC complex; and the second signal as the interaction of the T cell and the antigen-presenting cell via co-stimulatory ligands.

Within the context of this well-understood molecular mechanism, it is considered important to establish the meaning of the phrase "peptide-specific T cell" as understood by a skilled person in the immunological arts during the period 1993-1996. A search on Google Scholar (scholar.google.com) for academic references with [peptide specific "T cell"] in the title reveals 72 citations, indicating that the term 'peptide-specific' was commonly used to describe the mechanism by which T cells respond to their 'cognate' antigens. Ahmed *et al.* (Science 272:54-60, 1996) frequently refer to 'antigen-specific T cells' in their seminal review, and for example, on page 55, left column, describe 'antigen-specific $CD8^+$ T cells during primary and secondary responses to an MHC class I antigen.' Similarly, Kearney *et al.* (Immunity 1:327-339, 1994) frequently refer to 'anti-

gen-specific T cells' and 'peptide-specific T cells' when discussing the mechanism of inducing long-lasting immunity.

In marked contrast, a search in Google Scholar for citations published during the same time period and containing [phytohemagglutinin specific "T cell"] in the title revealed one citation, the word 'specific' in this case referring not to the mode of action of PHA on T cells, but to the characteristics of an antibody molecule (viz. "Specific IgG autoantibodies . . . phytohemagglutinin-activated T cells", Wolf-Levin *et al.*, J. Immunol. 151:5864-5877, 1993).

It is clear from this review of the literature that the action of PHA on T cells was not referred to as 'specific' in the immunological arts in the relevant time period. Consequently, one of ordinary skill in the art would have been unlikely to consider any aspect of the nature of the binding of PHA to the T cell surface as being 'peptide-specific.' As noted above, the process of activation of T cells in response to proteinaceous antigens, and their subsequent proliferation and differentiation into effector T cells – a process which represents one of the primary cell-mediated immune responses against a variety of foreign antigens *in vivo* in mammals – is highly specific in molecular terms, and is referred to as such in the art-related literature in and around 1996.

Applicants conclude that one of ordinary skill in the art would have considered any reference to the phrase 'peptide-specific' in the context of the *in vitro* responses of T cells which have been pre-sensitized *in vivo* to antigens as excluding phenomena pertaining to aspects of the molecular interaction of PHA on these cells *in vitro*, except perhaps when PHA is a cognate antigen. It is worth of emphasis that PHA was used as a non-specific mitogen in the Hagiwara citation referred to below; it was not employed as a cognate antigen.

B. The Phrase "Presenting to the T Cells"

The Examiner maintains the view that "stimulating the cells with a 1:100 dilution of phytohemagglutinin" is equivalent to "presenting to the T cells a T cell-activating peptide" (page 7, lines 19-20, item b, of the Action). As a result, it is pertinent to esta-

blish the true meaning of the phrase "presenting to the T cells a T cell-activating peptide" as it would have been understood by one of ordinary skill in the art.

A search on Google Scholar (scholar.google.com) for academic references with [presenting antigen "T cell"] in the title during the period 1993-1996 reveals 65 citations, indicating that the term 'antigen-presenting' was commonly used to describe the mechanism by which antigens were 'presented' to antigen-specific receptors on T cells (generally via MHC molecules). The textbook *Immunobiology, 6th Ed.* (Janeway *et al.*, Garland Science, 2005) provides the following definitions in its glossary:

> "Antigen presentation" describes the display of antigen as peptide fragments bound to MHC molecules on the surface of the cell. T cells recognize antigen when it is presented in this way.
>
> "Antigen-presenting cells" are highly specialized cells that can bind to MHC molecules for presentation to T cells.

Applicants' invention embodies the above concepts, in that it involves 'antigen presentation' of a T cell-activating peptide to effector T cells via antigen-presenting cells. As noted above, it was well established by 1996 that this occurred via the process of 'presentation' of the peptide by human MHC (i.e., HLA) molecules located on the surface of antigen-presenting cells, to antigen-specific receptors on the T cell surface.

In marked contrast, a search on Google Scholar for citations containing [presenting phytohemagglutinin "T cell"] in the title revealed no citations.

This brief exercise indicates that the word 'presenting' in the immunological art of the time was not used in the context of the action of PHA on T cells. The reason is evident: when it is not a 'cognate' antigen, PHA is not bound to T cells via this molecular mechanism *in vitro*. It follows that a skilled person will interpret any reference to the word 'presenting' in the context of the interaction of T cells with their cognate antigens as excluding any art pertaining to aspects of the molecular interaction of PHA on these cells *in vitro*.

C. Conditions Required for Activation of Specific T Cells

Dennis Klinman was the co-author of a major review of the ELISPOT technique in 1994 entitled "ELISPOT Assay to Detect Cytokine-Secreting Murine and Human Cells" in *Current Protocols in Immunology*, Units 6.19; already made of record. The Examiner has already acknowledged that "the referenced protocols in Klinman suggested the incubation of T cells with a T-cell activating peptide for several days." To emphasize this point, Unit 3.12 of *Current Protocols in Immunology* (already made of record and to which Klinman refers in respect of T cell activation) recommends 'Basic Protocol 2' which "describes the induction of a T cell proliferative response to soluble protein antigens . . . against which the animal has been primed *in vivo*." Basic Protocol 2 advocates an incubation time of two to four days.

In support of Klinman's teachings on various aspects of ELISPOT technology, numerous other references in the period 1993 - 1996 used long incubation times to measure antigen-specific T cell responses. For example, reference 22 (Clerici *et al.*, Science 262:1721-1724, 1993) and reference 40 (Clerici *et al.*, J. Clin. Invest. 93:768-775, 1994), which were cited by Hagiwara, incubated PBMCs with specific antigens for seven days. This is consistent with the protocol of Surcel further discussed below. In that paper, the authors actually state in the discussion section, "Conventionally, IFN-γ is measured in 5-7 days culture supernatants."

In summary, when Applicants' invention was made, there was an overwhelming body of literature recommending long-term *in vitro* incubations (of the order of several days) for stimulating T cells *in vitro* with their cognate antigens, a 'prejudice in the art' which one of ordinary skill in the art, who is expected to be thinking along the lines of conventional wisdom therein, is very likely to have adhered to at the time.

Scope of Applicants' Claimed Invention

Applicants' specification frequently refers to peptide-specific T cells, antigen-specific T cells, and to 'enumerating precursor effector T cells.' Further, the examples of the instant application describe results using peptide epitopes which are MHC-restricted (e.g., Table 1, Example 1), showing that the mechanism of T cell activation in Appli-

cants' invention is critically dependent on the interaction between human MHC molecules 'loaded' with a given peptide and antigen-specific receptors on effector T cells.

In view of the above, Applicants believe that it is entirely evident to the skilled artisan that the scope of the instant invention is limited to a method which measures the responses of T cells *in vitro* to their cognate antigens via these highly specific mechanisms, and thus excludes any consideration of the mechanisms of non-specific binding *in vitro* of proteinaceous substances, such as PHA mitogen, to numerous types of molecule situated on a T cell's surface. Furthermore, mitogens such as PHA are not dependent on pre-sensitization of T cells. Therefore, PHA would not have the ability to stimulate "interferon-γ release by only those T cells that have been pre-sensitized *in vivo* to the T cell-activating peptide."

The Action of PHA

Applicants' first Amendment made the point that it was well known around 1994 that PHA non-specifically cross-links human T cell surface membrane glycoproteins, including the T-cell antigen receptor and possibly the CD3 molecule, to induce polyclonal activation and agglutination of T cells. In suggesting that the PHA experiments outlined in Hagiwara would motivate one of ordinary skill in the art to apply the published incubation conditions to Miyahira's methodology, thus giving rise to Applicants' invention, the Examiner's assertions fly in the face of a prolific number of research papers characterizing, on the one hand, the effects of PHA on lymphocytes, and on the other, the mechanisms of activation of T cells by their specific cognate antigens *in vitro*.

In 1960, Peter Nowell first indicated (Cancer Research, 20:462-466, 1960) that PHA was an initiator of mitotic activity. Nowell did not recognize that some of the cells responding to PHA are small lymphocytes, although this was confirmed shortly afterwards by Gowans *et al.* (*CIBA Foundation Study Group No. 10: Biological Activity of the Leukocyte*, Boston: Little, Brown, pp. 32-40, 1961). A subsequent paper by Epstein *et al.* (J. Clin. Invest. 50:744-753, 1971; copy attached) shows the time course of induction of interferon in lymphocytes following PHA stimulation in Figure 2, and it is clear that this cytokine is rapidly induced within the first several hours of incubation. Later, Faguet

(J. Biol. Chem. 252:2095-2100, 1977; copy attached) showed that PHA binds to human lymphocytes within 30 minutes of incubation, and refers to other work in its Discussion section confirming that biochemical and metabolic changes are induced within one hour of exposure to PHA mitogen.

In summary, the induction kinetics of interferon in lymphocytes by PHA *in vitro* had been known for approximately 20 years at the time of the instant invention. Yet by 1994, the date of Klinman's review paper on ELISPOT techniques, no one had taken account of this wealth of data characterizing PHA action and, as a result, suggested the application of short incubation times to facilitate activation of T cells by their cognate antigens *in vitro*. This situation strongly supports the view that the mode of action of PHA *in vitro* was not considered to be germane to the mechanisms of activation of the primary immune response *in vitro*.

Given that Hagiwara itself teaches nothing new over the already existing art in relation to the mode of action of PHA (see below), it would therefore be extremely unlikely that one of ordinary skill in the art would have considered adopting Hagiwara's 'PHA incubation strategy' to trigger a primary immune response in T cells *in vitro*.

Further, the Examiner alleges that "the mechanism of how the T cells are activated is irrelevant" (page 15, lines 13-14, of the Action). Applicants strongly dispute this conclusion for reasons set out above, and summarize our position here. The state of the prior art discussed above indicates that one of ordinary skill in the art would, in 1996, have known about the different mechanisms of action of PHA and cognate antigens *in vitro*. Such a person reviewing the prior art in order to identify technical approaches which might address a given problem would be influenced by the particular immunological mechanism(s) being addressed by the technical approaches. If a given approach does not in any way address the mechanism underlying the problem at hand, that person would be unlikely to apply it to the problem.

Before addressing more precisely the actual combinations of documents relied upon by the Examiner, Applicants wish to firstly examine the precise disclosures of each of those documents individually in more detail.

Contents of the disclosure of 'Hagiwara'

With reference to the Examiner's response to the arguments in the Action, Applicants wish to point out a factual error. On numerous occasions, the Examiner attributes the word 'fresh' to the Hagiwara citation, and implies that this citation teaches that the use of fresh cells will reduce or minimize 'inconsistent results.' In fact, the word 'fresh' is not used in the entire text of this citation, and there is no discussion of the value of directly applying PBMCs to the antibody-coated plates. Consequently, Applicants submit that there is no teaching in Hagiwara as to the merits of using fresh cells for *in vitro* incubations, and certainly not in the context of an ELISPOT assay employing T cell-activating peptides. The objectives of Hagiwara's work are characterized, and then their results are analyzed in the context of their use of PHA.

The thrust of Hagiwara is to examine the effect of HIV infection in humans on the production of Type 1 (e.g., IL-2 and IFN-γ) and Type 2 (e.g., IL-4 and IL-10) cytokines *in vivo* by PBMCs. Although not unambiguously stated, the work appears to have been undertaken in efforts to throw some light on previous and conflicting reports describing various changes in cytokine production in PBMCs following HIV infection. The study concludes that, although the number of PBMCs secreting Type 1 and 2 cytokines was reduced in HIV patients, there was no correlation between the magnitude of these reductions and disease severity.

As expanded upon further below, the PHA mitogen method of Hagiwara will evidently give a measure of the level of any PBMCs which are capable of activation by the mitogen and thereby produce a detected cytokine. Hagiwara and colleagues found that this was 2- to 3-fold **lower** in HIV patients compared to healthy individuals (see the results under the heading "frequency of cytokine-secreting cells in HIV-infected patients"). In contrast, a method of Applicants' invention would very probably find HIV specific effector T cells in PBMCs from HIV patients but not in individuals with no HIV present in view of the use of T cell-activating peptide precisely to target activation of the effector T cells of interest. **The objectives, and outcome, of Hagiwara were therefore completely different from those of Applicants' invention.**

At a practical level, the work measured the production of cytokines in the absence of any antigen addition *in vitro*, all assays being incubated for six hours. There appears to be no immunological basis to support the use of this incubation time. The first mention of a reason for introducing the mitogen PHA in some of these experiments is in the Materials and Methods section where it is stated, "Other investigators analyzed the effect of *in vitro* mitogen stimulation on cytokine production by PBMC. We therefore added the T cell mitogen PHA during the ELISPOT assay" (page 128, last 8 lines). This statement, it appears, is the sole rationale for using PHA in this work. One can only reasonably assume that cells were incubated with PHA for six hours in order to obtain a direct comparison with the 'non-antigen' assays. In summary, apart from these unproven assertions, there is no teaching of any rationale for using the specified incubation time, whether or not PHA was added to stimulate the cells.

Whereas Applicants' invention enumerates a particular subset of T cells that respond to a specific antigen *in vitro* following pre-sensitization of those T cells to that antigen *in vivo*, Hagiwara's PHA experiments measure the general level of a non-specific response of a population of cells in PBMCs that are activated by a mitogen. The results bear absolutely no relation to the enumeration of antigen-specific effector T cells, because:

a) PHA is not employed as a cognate antigen,
b) PHA does not act via the primary immune response mechanism,
c) different T cell types (e.g., naïve, memory, effector) would all be expected to secrete cytokines in response to PHA, and
d) as explained by Romagnani (Immunol. Rev. 140:74-92, 1994; see also reference 42 in Hagiwara), PHA stimulates cytokine production in cells other than T cells (see page 76), for example, in macrophages, B cells and NK cells, thus rendering it impossible to attribute the measured response to an action of PHA on T cells alone; Hagiwara seems to be fully aware of this latter point, because, as indicated on page 131, "This approach . . . identifies all cytokine-secreting cells in the immune milieu."

Yet, even in the light of all of the above, the Examiner continues to assert that Hagiwara's technique is a "method of assay in which peptide-specific effector T-cells

are enumerated" (page 7, line 14, of the Action) and then proceeds to set out four steps a) to d) describing this protocol, aligning Hagiwara's 'steps' with the steps set out in claim 40 of Applicants' invention. This attempted correlation is simply not valid from an immunological standpoint. In particular, "stimulating the cells with . . . phytohemagglutinin" cannot reasonably be considered to be equivalent to the phrase "presenting to the T cells a T cell-activating peptide," given the strict immunological meaning of the term 'presenting' (see above).

At this point, it would seem worth highlighting that the senior author of Hagiwara is Dennis Klinman, an author of a review of protocols related to the ELISPOT technique (Klinman *et al.*, 1994; already made of record) that was acknowledged by the Examiner to suggest the use of long incubation times to stimulate T cells via their cognate antigens. Consequently, if Hagiwara had identified a new method in the context of generating an *in vitro* response in T cells pre-sensitized *in vivo* to cognate antigens, one might have expected something to be said about this in Hagiwara or elsewhere. It is submitted that the fact that a recognized ELISPOT assay expert such as Klinman did not make the connections that the Examiner is trying to force, is a good indicator that the Examiner is employing thinking that would never have been employed by one of ordinary skill in the art at the time of invention.

In summary, Applicants submit that in asserting that Hagiwara makes certain 'suggestions' to one of ordinary skill in the art, the Examiner has improperly employed hindsight in order to interpret Hagiwara's disclosure. Applicants additionally submit that the value of 'fresh cells' in relation to detection of effector T cells is not taught in Hagiwara. Finally, Applicants submit that there is no teaching in Hagiwara which would motivate one of ordinary skill in the art to interpret Hagiwara's incubation protocol using PHA as being a feasible approach to achieve the enumeration of a particular subset of T cells that respond to a specific (cognate) antigen *in vitro* following the pre-sensitization of those T cells to that antigen *in vivo*. And based on a knowledge of other, earlier art characterizing the 'blanket' nature of PHA action, one of ordinary skill in the art would not have been encouraged by using Hagiwara's protocol to <u>specifically</u> activate effector

T cells *in vitro*, which were pre-sensitized *in vivo*. Applicants discuss the merits of Hagiwara's incubation protocol in the absence of PHA below.

The disclosure of 'Miyahira'

Applicants have already pointed out that Miyahira's ELISPOT results equate with those obtained using LDA, thus leading a skilled person away from the idea of using Miyahira's protocols to detect cytokine responses in antigen-specific effector T cells.

The disclosure of 'Surcel'

The Examiner indicated, "Surcel's method is intended for measuring effector T cells" (page 12 of the Action). Applicants dispute this incorrect conclusion. Firstly, Surcel does not make such a statement explicitly. Secondly, the Examiner already acknowledged that (i) "Surcel's measurement of IFN-gamma producing T cells involves incubation of T cells . . . for, what is reasonably deduced from the context of the protocol, 72 hours" and (ii) "The incubation of T cells with T cell activating peptide for 72 hours would allow memory T cells to proliferate." Applicants agree with this latter assessment.

In the first Amendment at page 9, it was pointed out that, typically, a human cell in culture divides every 24 hours. One of ordinary skill in the art would be aware of this fact. In consequence, Surcel cannot be measuring effector T cell activity exclusively, and any interpretation of results obtained will necessarily be complex since the data will most probably have been generated by effector T cells, memory T cells, and even naïve T cells.

To confuse matters further, Surcel also concludes that "the numbers of IFN-γ-secreting cells did not differ significantly between patients and controls" (i.e., TB patients and controls at Discussion, second paragraph, lines 16-17). This statement must surely lead one of ordinary skill in the art away from the idea of using Surcel's technical approach in efforts to establish *in vitro* methods of differentially detecting infectious disease *in vivo*.

The disclosure of 'Sørensen'

The Examiner has already conveniently pointed out that "Sørensen discloses the discovery of a low-molecular-mass T-cell antigen secreted by *Mycobacterium tuberculosis*" and that this reference also discloses that "ESAT-6 elicited a high release of IFN-γ from T cells isolated from memory-immune mice challenged with *M. tuberculosis*." Applicants agree with the Examiner's statement. But her use of Sørensen is contested. The Examiner's selected combinations of the above citations are now discussed below.

Claims 40 and 43-50 were rejected under Section 103(a) as allegedly unpatentable over Miyahara *et al.* (J. Immunol. Meth. 181:45-54, 1995; hereinafter "Miyahara") in view of Hagiwara *et al.* (AIDS Res. Hum. Retrovir. 12:127-133, 1996; hereinafter Hagiwara). Applicants traverse for the following reasons.

A. Combined Documents Teach Only Concept 5 of the Instant Invention

The Examiner contends that the instant invention is obvious over Hagiwara in view of Miyahira. Initially, taking a broad perspective, Applicants have already pointed out that their invention requires the integration and interplay of seven concepts (see the diagram summarizing these Concepts, and their interrelationship, attached as Annex I):

Concept 1 – The presence of antigen is essential to maintain population of circulating activated effector T cells *in vivo*.

Concept 2 – Effector T cells respond to antigen *in vitro* without need to proliferate or differentiate.

Concept 3 – Memory T cells only respond *in vitro* following proliferation (generally after 24 hours of *in vitro* stimulation with antigen).

Concept 4 – In view of Concepts 1-3 above, selective detection of pathogen-specific effector T cells could provide a dynamic surrogate marker for the presence of, or recent infection by, pathogen in a host.

Concept 5 – ELISPOT is a sensitive method, and able to enumerate responsive peptide-specific T cells *in vitro*.

Concept 6 – ELISPOT is also sufficiently sensitive to detect effector T cells above background levels before any interference from memory progeny cells occurs at later incubation times.

Concept 7 – In view of anticipated short *in vitro* incubation times, fresh cells should be introduced directly into ELISPOT wells.

At best, Miyahira teaches Concept 5, that is, that ELISPOT is a sensitive method. Hagiwara teaches none of the key concepts outlined above and diagrammed in Annex I. Therefore, Applicants submit that, as of 1996, one of ordinary skill in the art would have needed to have been in possession of at least six scientific ideas, in addition to Miyahira's teaching of concept 5, in order to gain the insight of Applicants' invention. As a starting point, a key concept that the effector T cell could potentially provide a dynamic marker for intracellular pathogens (Concept 4) was not known to be publicly available prior to Applicants' invention. This fact is not changed by attempting to combine the teachings of Hagiwara and Miyahira.

By way of contrast, Applicants were motivated to carry out the practical work necessary to prove the invention (Concepts 5-7), because this work was preceded by the realization that effector cells might potentially represent a dynamic marker for the presence *in vivo* of infectious agents, a realization which first came from carrying out the studies reviewed in Example 1 but only published after the filing date of Applicants' priority document. This initial innovation then led to a valuable application in disease monitoring having a real practical utility.

Finally, Applicants refer briefly to Concept 7: namely 'the introduction of fresh cells to the ELISPOT wells.' As already noted above, Applicants maintain that Hagiwara does not teach the value of fresh cells to the skilled person. Even if this was not the case, however, and the skilled person was indeed motivated to combine Hagiwara with Miyahira, the claimed invention would not have been obtained. In fact, these two citations would have collectively taught only Concepts 5 and 7 from a total of seven concepts needed to arrive at the instant invention.

B. Examiner's Interpretion of the Text in Hagiwara

In summarizing her position, the Examiner indicates on page 15 of the Action, "In response to Applicant's arguments, the combination of references results in Miyahira's ELIspot, as disclosed, with the modification/incorporation of Hagiwara's suggestion to use fresh T cells that are not incubated *in vitro* to measure effector T cell function, and a 6 hour incubation time for activation of effector T cells (Hagiwara's method, page 128)."

In the context of the above statement, Applicants emphasize that:

1) Hagiwara does NOT in any way suggest using fresh T cells – see above;
2) Hagiwara does NOT suggest in any way the use of cells, fresh or otherwise, that are not incubated *in vitro*, to measure effector T cell function;
3) Neither the phrase 'effector T cell' nor 'effector T cell function' exists anywhere in the text of Hagiwara; and
4) Hagiwara does NOT suggest use of a 6 hour activation time to activate effector cells.

Applicants would suggest that the Examiner, with the benefit of hindsight developed over approximately ten years, has over interpreted Hagiwara's disclosure and inflated its teachings over their plain meaning, and that one of ordinary skill in the art, at a time of the invention, would not have drawn the same conclusions.

C. Lack of Basis for Motivating the Skilled Person to use the Approach

The main focus of Hagiwara is on assays performed without PHA stimulation. But referring to previous 'third party' work, Hagiwara indicates that "divergent results were generally obtained by studying PBMC that had been cultured and stimulated *in vitro*" (see Discussion, bottom of paragraph 3, page 131). It is clear from the text that these results include experiments carried out with mitogens including PHA (see references 22, 26 and 40 cited by Hagiwara). Accordingly, this statement teaches away from the use of PHA to stimulate cultured cells *in vitro* in efforts to achieve consistent results. Nevertheless, the Examiner has implied (page 7, line 11, to page 8, line 9, of the Action) that since such *in vitro* results were divergent, Hagiwara chose an alternative strategy which involved the induction of PBMCs by PHA.

Applicants submit that such an argument is specious. In fact, the alternative strategy was "to study cells actively secreting cytokines *in vivo*." This is supported by a subsequent comment in paragraph 5, namely "This approach is uniquely suited to determining whether a physiologically relevant shift from type 1 to type 2 cytokine production . . ., since it identifies all cytokine-secreting cells in the immune milieu rather than focussing on cell . . . responsive to stimulation *in vitro*." In summary, Hagiwara recommends to the skilled artisan an 'alternative approach', which is to measure cytokine production *in vivo* without addition of stimulating agents *in vitro*, in efforts to correlate infectious disease status and immune status, and accordingly leads the skilled person away from any consideration of using PHA to induce cytokines if consistent results are required.

The Examiner also indicated that the "same concept applies to Miyahira's ELIspot" (page 7, line 8, of Action). Given the context of this statement, the 'concept' referred to can only be interpreted to mean that of 'divergence of results' in samples from patients with infectious disease; that is, marked variations in experimental results between different scientific teams following *in vitro* incubation of cells with antigens/ mitogens. The thrust of Miyahira's work was to examine the induction of cytokines in T cells 'after their stimulation with antigen.' Accordingly, Miyahira incubates cells with antigens in culture for long periods – that is, performs precisely the type of experiment that Hagiwara considers would give rise to divergent results. As was stated in our previous response, Miyahira teaches precisely what Hagiwara advises against.

It follows that one of ordinary skill in the art would not have been persuaded by Hagiwara to apply the incubation times used for PHA to Miyahira, because it would lead to divergent results. Hagiwara would, if anything, persuade the skilled artisan to choose an alternative strategy of studying cells actively secreting cytokines *in vivo*. And herein lies the dilemma. If the skilled person had wished to examine the response of T cells to antigens *in vitro*, as exemplified in Miyahira, then the results obtained using Hagiwara's proposed alternative strategy would have been incompatible since it lacks any synergy, because the work does not employ antigens; by the same token, one of ordinary skill in the art would have been dissuaded from using Hagiwara's protocol involving PHA, because Hagiwara teaches that this would lead to divergent results.

Further, the Examiner states, "One of ordinary skill in the art would have been motivated to reduce inconsistent results by using fresh T cells" (page 14, lines 13-14, of Action). This cannot be true, based on a skilled person's perception of the above dilemma: the alternative strategy does not use antigens and the PHA strategy leads to inconsistent (i.e., divergent) results.

In summary, Applicants submit that one of ordinary skill in the art, in the course of applying even the most basic logic, would be dissuaded from incorporating Hagiwara's teachings into Miyahira's method, and particularly so given the nature of the then existing state of the art. In particular, this is because of two conflicting and confusing messages in Hagiwara:

a) Teaching 1 – namely to study cells secreting cytokines *in vivo* in order to assess infectious disease status, does not involve addition of added antigen, and so provides no basis on which it could complement Miyahira and
b) Teaching 2 – to incubate cells with PHA, which is discouraged because it is considered to lead to inconsistent results.

The Examiner has overlooked this internal conflict which teaches away from Applicants' invention and lead one of ordinary skill in the art away from the invention of the pending claims.

D. Dynamics of T Cell Responses to Antigens in PBMCs

Effector T cells represent a very small percentage of total T cells in the PBMC fraction. For example, Kalams *et al.* (J. Exp. Med. 179:1261-1271, 1994; copy attached) indicate that the frequency of the effector CTL population specific for a particular peptide of HIV is 1/1016 in patients with HIV, thus representing a very small percentage of total cells in PBMCs. This low level represents one of the factors which contributes to the difficulty of detecting these T cells. Hence, a key concept to be proved to arrive at the invention was that the ELISPOT technique is sufficiently sensitive to detect effector T cells above background prior to any interference from memory progeny cells (Concept 6).

By dramatic contrast, PHA is known to activate a very high percentage of T Cells, as indicated in our previous response.

Taking these facts alone, one of ordinary skill in immunology is extremely unlikely to conclude that the conditions used to stimulate PBMCs with PHA in Hagiwara could have value when applied to Miyahira, and that this would enable exclusive detection of effector T cells. To put it another way, on what basis might one of ordinary skill conclude that the six-hour incubation protocol for PHA used in Hagiwara, during which virtually all cells are responsive, could potentially be of use for detecting a particular T cell population comprising less than 0.01% of the total? Applicants submit that no such basis exists. And in the light of Romagnani (see above), who notes that PHA also stimulates cell types other than T cells, Hagiwara's ability to motivate one of ordinary skill in the art to sensibly tackle the hypothetical task at hand must surely be remote.

It is reiterated that one of the authors of the Hagiwara citation is Dennis Klinman, a co-author of a major review of ELISPOT protocols in 1994 which recommends that long incubation times be used to stimulate T cells via their cognate antigens. It is very likely, therefore, that if Hagiwara and coworkers had identified a new method in the context of generating an *in vitro* response in T cells pre-sensitized *in vivo* to cognate antigens, it would have been pointed out in the cited reference. The reality is that the protocols in Hagiwara have little, if any, relevance to the achievement of this latter objective.

Claims 40-43 and 45-48 were rejected under Section 103(a) as allegedly unpatentable over Surcel *et al.* (Immunol. 81:171-176, 1994; hereinafter "Surcel") in view of Sørensen *et al.* (Infect. Immun. 63:1710-1717, 1995; hereinafter "Sørensen") and Hagiwara. Applicants traverse for the following reasons.

A. Combined Documents Do Not Teach Key Concepts For Attainment Of The Invention

The Examiner suggests that the instant invention is obvious over Surcel in view of Sørensen and Hagiwara.

As noted above, the instant invention requires the integration and interplay of seven Concepts. Surcel clearly teaches that the ELISPOT technique is a sensitive method, but neither Sørensen nor Hagiwara teach any of the key Concepts. The combination of these three citations again leaves one of ordinary skill in the art needing to input key Concepts which simply were not part of the common general knowledge, giving rise to the idea that the effector T cell could potentially provide a dynamic marker for intracellular pathogens (Concept 4), the Concept that came to the inventors from the studies of Example 1 but which was not publicly available at the filing date of the priority document.

As a result, absent a comprehension of the relevant Concepts, one of ordinary skill in the art would have no motivation to combine the teachings of Surcel, Sørensen and Hagiwara, and would have needed to add still more in terms of creative thinking akin to inventive ability, in order to attain the instant invention. In contrast, Applicants were motivated to carry out the practical work necessary to prove the feasibility of their invention (Concepts 5-7), because this work was preceded by the realization that T effector cells might potentially represent a dynamic marker for the presence *in vivo* of infectious agents (Concepts 1-4). This initial innovative thinking then led to a valuable application in disease monitoring having a real practical utility.

Surcel's authors were experienced researchers in the TB field, but were not focusing on detection solely of effector cells as a marker of infection. Indeed, their experimental design meant that they were attempting to detect changes in T cell activity linked to infection above high 'control' values representing a response from predominantly memory progeny cells. As regards finding a means to either diagnose or monitor TB infection, they were missing important concepts needed to turn failure into success, for which the later Hagiwara studies, either without antigen or with the mitogen PHA, could be of no interest or assistance.

B. Lack of Basis For Motivating The Skilled Person To Use The Approach

The Examiner alleges that "it would have been obvious to use ESAT-6 as the activating peptide in Surcel's ELIspot method." Applicants agree with the Examiner on

her point because Sørensen discovered a new *M. tuberculosis* antigen, the use of which would have probably complemented the work of Surcel, who selected, for example, *M. tuberculosis* 38,000 and 19,000 MW antigen preparations, and peptides derived therefrom, to stimulate T cells. In short, it would have been obvious to the skilled person to test another immunodominant antigen.

But as stated in Applicants previous response, even if ESAT-6 proteins and/or peptide fragments were used as the activating antigen in Surcel's ELISPOT method, it would not result in Applicants' invention. Nor would there be any motivation leading to Applicants' invention because Surcel's ELISPOT method does not distinguish between effector T cells and memory T cells.

Furthermore, one of ordinary skill in the art would be no more motivated to apply Hagiwara to the combination of Surcel and Sørensen, than to Surcel alone. If one of ordinary skill in the art wished to examine the response of T cells to antigens (especially ESAT-6) *in vitro*, as exemplified by a combination of Surcel and Sørensen, then the results obtained using Hagiwara's proposed 'alternative' strategy are irrelevant, because the work does not employ antigens. And the use of Hagiwara's protocol involving PHA is discouraged, because it would lead to 'divergent' results. These teachings are hardly of the quality needed to motivate an immunologist thinking along the lines of the conventional wisdom in the art to apply either of them to Surcel / Sørensen.

In summary, Applicants submit that one of ordinary skill in the art employing even the most fundamental logic would not be persuaded to incorporate Hagiwara's disclosure into Surcel's method using Sørensen's antigen, and particularly so given the nature of the then existing state of the art.

The Examiner has noted additionally the reference to non-sterile conditions in claim 45. This does apply to usage of the invention, but it is the Concepts as set out in Annex 1 which are clearly of paramount importance to attaining the invention. It is the failure of any combination of cited documents to teach these *in toto* which cannot be overlooked, and supports progress of the art amounting to a true inventive step. More-

over, this conclusion is consistent with what are considered to be secondary indicators of invention as outlined below.

Applicants' Invention is a Major Immunological Advance and a Commercial Success

The commercial success of, and unexpected advantages provided by Applicants' invention, strongly support the case for non-obviousness of claim 40.

Applicants' invention has had a major impact on science and medicine in recent years, since it revolutionized the way in which immunologists measure T cell responses. It is the most sensitive, robust and widely used quantitative technique for measuring antigen- and peptide-specific effector T cell responses in humans, as well as animal models. The inventors' peer-reviewed publication based on the studies of Example 1 (Lalvani *et al.*, J. Exp. Med. 186:859-865, 1997; already made of record) which first made the concepts behind the invention public, has been cited numerous times in the immunological literature. It is fair to say that, at the present time, immunologists enumerate antigen-specific effector T cells directly *ex vivo* as a matter of course, in preference to assays that depend on cell proliferation. The technique represents one of the three main techniques used for *ex vivo* enumeration of T cells, alongside tetramer staining and intracellular cytokine staining with FACS analysis.

The T-SPOT.*TB* test for diagnosing TB, which is encompassed by the pending claims, was launched into Europe by Oxford Immunotec Limited, UK in 2005, and is already having a major impact on a global disease that is a leading cause of death worldwide. The effective control and eventual elimination of tuberculosis from developed countries is a public health priority. The realization of this goal hinges on diagnosing and treating people with latent tuberculosis infection (LTBI). This strategy is hampered by the limitations of the century-old tuberculin skin test (TST). The T-SPOT.*TB* test is arguably the most significant advance for diagnosing LTBI since the development of the TST. It was the recognition that one could use the enumeration of effector T cells *ex vivo* to diagnose infection with an intracellular pathogen such as *M. tuberculosis* that made this landmark advance possible.

Finally, the instant invention is the subject of a granted patent in Europe, which was maintained as EP 0941478 B2 (published June 6, 2007) following opposition proceedings at the European Patent Office (EPO). This was in the face of scrutiny by two company opponents with technical experts in the field and a three-examiner Opposition Division aware of all the documents relied on by the Examiner in this application. Yet Hagiwara is not even mentioned in the Opposition Decision, a copy of which was previously provided for review by the Examiner, entirely in keeping with the conclusion now made that it is not a key element of prior art providing any sort of stepping stone to the invention, whether considered with Miyahira and/or Surcel and Sørensen.

Withdrawal of the Section 103 rejections is requested because Applicants' claims would not have been obvious to the ordinarily skilled artisan at the time they made their invention.

Having fully responded to all of the pending objections and rejections contained in this Office Action, Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

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